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ORIGINAL ARTICLE

'Okra' *Hibiscus esculentus* L.: A study of its hepatoprotective activity

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Abstract In the present study, an attempt has been made to validate the claimed uses of 'Okra' *Hibiscus esculentus* in liver diseases. The preventive action of ethanolic extract of okra (EEO) against liver injury was evaluated in rodents using carbon tetrachloride-induced hepatotoxicity model. EEO, at 250 and 500 mg/kg body weight, exerted significant dose-dependent hepatoprotection by decreasing the CCl_4-induced elevation of serum SGOT, SGPT, ALP, GGT, cholesterol, triglycerides and malondialdehyde (MDA) non-protein sulfhydryls (NP-SH) and total protein (TP) levels in the liver tissue. A significant reduction was also observed in pentobarbital-induced sleeping time in mice. The hepatoprotective and antioxidant activities of the extract are being comparable to standard silymarin. These findings were supported by histological assessment of the liver biopsy. The ability of okra extract to protect chemically induced liver damage may be attributed to its potent antioxidant property.

1. Introduction

Liver disease is considered to be a serious health problem as liver is an important organ for the detoxification and deposition of endogenous substances. Administration of vaccines, corticosteroids and antiviral drugs has been found to be the best remedial option for the treatment of liver diseases in conventional medicine. These treatments are not free from serious and adverse effects, especially when given for a prolonged period (Yang et al., 2008). Therefore, herbs, vegetables and medicinal plant products with improved effectiveness and safety profiles are needed as a substitute for chemical drugs. It has been recognized that vegetables and fruits are essential and well balanced diet required for a healthy living. It has also been recognized that high consumption of some vegetables and fruits are beneficial to health and in combating the onset of cancer, coronary diseases, inflammation, arthritis, immune system decline, liver diseases and brain dysfunctions (Shui and Peng, 2004).

Okra, *Hibiscus esculentus* L. (Syn. *Abelmoschusesculentus*) (EEO) is one of the members of the Malvaceae family. It is known by many names, Lady's finger, Bamyah and Bhindi;

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which is eaten raw as well as cooked. Egyptians believed that eating okra prevents the development of urinary calculi (Kamal, 1975). In folkloric practice fresh tender pods were given to cure constipation, leucorrhea, spermatorrhea, diabetes and jaundice (Chopra et al., 1956). Mucilage is also used as soothing emollient medicine in the treatment of diarrhea, dysentery and gastric ulcer. A cupful of mucilage mixed with a ripe banana is given as a tonic-food during the treatment of colitis, cystitis, hepatitis and jaundice (Chopra et al., 1956; Aman, 1969). A gastroprotective effect of the methanolic extract of okra in ethanol-induced gastric ulcer in rats was reported (Gurbuz et al., 2003). Recently, some major antioxidant quercetin derivatives were identified and isolated from okra (Shui and Peng, 2004). Fatty and amino acids and minerals, especially zinc contents (Hirose et al., 2003), and lepidimoide have been isolated from okra mucilage, which has multiple functions in the growth and development of plants (Cook et al., 2000). Based on its folkloric use and reported antioxidant constituents, the present study was carried out to evaluate the protective effect of the ethanolic extract of okra (EEO) against carbon tetrachloride-induced hepatocellular injury in rats.

2. Materials and methods

2.1. Drugs and chemicals

Carbon tetrachloride (CCl_4) was procured from E. Merck, Germany. Silymarin was purchased from Sigma Co. (St. Louis, MO). Enzymatic *in vitro* diagnostic kits were obtained from Roche Diagnostics Corporation, UK. All other chemicals used in this study were of analytical grade.

2.2. Plant material and preparation of extract

The fresh pods of 'Okra' *Hibiscus esculentus* were purchased from the local vegetable market in Riyadh and its identity was confirmed by expert taxonomist of the Department of Pharmacognosy, where a voucher specimen (No. 3608) of the plant has been kept in the Herbarium. Shade dried pods (500 g) were coarsely powdered and macerated in 3 l of 96% ethanol for 72 h using percolation method. The solvent was then removed at 40 °C under reduced pressure in a rotavapor. The extract was then suspended in distilled water before its administration.

2.3. Phytochemical screening

The preliminary qualitative phytochemical screening of okra was conducted for the presence and/or absence of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or triterpenes (Fransworth, 1966).

2.4. Animals

Wistar albino rats of either sex and approximately the same age (8–10 weeks), weighing 180–200 g, obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh, were used. Swiss albino mice were used for studying sleeping time and acute toxicity. The animals were kept at a constant temperature (22 ± 2 °C), humidity (55%) and 12 h light–dark conditions. The animals were then provided with Purina chow diet and drinking water *ad libitum*. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.5. Acute toxicity test

The acute toxicity test was performed in five groups 10 mice (five male and five female mice each). Okra extract was dissolved in distilled water and administered orally at various doses, ranging from 0.5, 1, 2, 4 and 8 g/kg. The animals were observed for clinical signs and symptoms of toxicity every 30 min up to 6 h on the first day and thereafter, everyday up to 7 days. The mortality occurring in each group was recorded (Al-Rehaily et al., 2002).

2.6. Carbon tetrachloride-induced liver toxicity

Rats were divided into five groups (1, 2, 3, 4 and 5) ($N = 6$ animals/group). Group 1 was kept as control (distilled water). Groups 2–5 received CCl_4 in liquid paraffin (1:1) at 1 ml/kg body weight intraperitoneally (IP) (Rafatullah et al., 2008). Group 2 was administered with only CCl_4. Groups 3 and 4 were treated with EEO at the doses of 250 and 500 mg/kg body weight, respectively, and group 5 was administered with silymarin at a dose of 10 mg/kg body weight orally. EEO treatment was started 10 days prior to CCl_4 administration and continued until the end of the experiment. The blood was collected by cardiac puncture after 24 h following CCl_4 administration, allowed to clot and the serum was separated. After blood collection, the animals were sacrificed using ether anesthesia. The liver was dissected out and used for biochemical studies and histological examination.

2.7. Estimation of marker enzymes and bilirubin

Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT) and bilirubin were determined using Reflotron® Plus Analyzer and Roche kits.

2.8. Estimation of lipid profile

The total cholesterol (Demacher and Hijamaus, 1980) and triglyceride (Foster and Dunn, 1973) levels were estimated in serum.

2.9. Determination of malondialdehyde (MDA)

The method reported by Utley et al. (1967) was followed. The liver tissues were removed and each tissue was homogenized in 0.15 M KCl (at 4 °C; Potter-Elvehjem type C homogenizer) to give a 10% w/v homogenate. Aliquots of homogenate (1 ml) were incubated at 37 °C for 3 h in a metabolic shaker. Then 1 ml of 10% aqueous trichloroacetic acid was added and mixed. The mixture was then centrifuged at 800*g* for 10 min. One milliliters of the supernatant was removed and mixed with 1 ml of 0.67% thiobarbituric acid and placed in a boiling water bath for 10 min. The mixture was cooled and diluted with 1 ml

distilled water. The absorbance of the solution was then read at 535 nm. The content of malondialdehyde (nmol/g wet tissue) was then calculated, by reference to a standard curve of malondialdehyde solution.

2.10. Estimation of non-protein sulfhydryls (NP-SH)

Hepatic non-protein sulfhydryls were measured according to the method of Sedlak and Lindasy (1968). The liver was homogenized in ice-cold 0.02 mmol/l ethylenediaminetetraacetic acid (EDTA). Aliquots of homogenates (5 ml) were mixed in test tubes with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 rpm. Two milliliters of supernatant mixed with 4 ml of 0.4 mol/l Tris buffer (pH 8.9). 0.1 ml of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured within 5 min of the addition of DTNB at 412 nm against a reagent blank.

2.11. Determination of total protein (TP)

Total protein was estimated by the kit method, supplied by Crescent Diagnostics, Jeddah, Saudi Arabia.

2.12. Histopathological evaluation

Liver tissue samples were fixed in neutral buffered formalin for 24 h. Sections of the liver tissue were histopathologically examined to study the hepatoprotective activity of EEO. The tissues were fixed in 10% buffered formalin and processed using a VIP tissue processor. The processed tissues were then embedded in paraffin blocks and sections of about 5 μm thickness were cut by employing an American optical rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures (Culling, 1974). The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema, and erosions using an arbitrary scale for severity assessment of these changes.

2.13. Sleeping time

The sleeping time in mice was measured using pentobarbital. Mice were divided into four groups of 10 animals each. Group I received the distilled water only; group II received CCl_4 only. Groups III and IV received EEO (250 and 500 mg/kg body weight). Thirty minutes later, the animals of groups II, III and IV were treated with pentobarbital (50 mg/kg I.P.). The time interval between the onset and the regaining of the righting reflex was measured as the sleeping time (Dandiya and Collumbine, 1959).

2.14. Statistical analysis

Values are given as arithmetic means ± standard error of the mean (SEM). Data were statistically analyzed by using one-way analysis of variance (ANOVA) followed by the Student's *t*-test.

3. Results

3.1. Phytochemical screening

The preliminary qualitative screening of okra pods, revealed the presence of flavonoid, tannins, anthracene, sterols and/or triterpenes.

3.2. Acute toxicity test

No toxicity symptoms were recorded. The LD_{50} value by oral route could not be determined as no lethality was observed up to 8 g/kg of the okra extract in mice.

3.3. Effect of EEO on marker enzymes in serum

The effect of protreatment of rats with ethanolic extract of okra on the CCl_4-induced elevation of the serum GOT, GPT, ALP and GGT are shown in Table 1. Administration of CCl_4 significantly elevated the release of GOT, GPT, ALP and GGT contents in serum ($P < 0.001$) as compared to normal control group. Pretreatment of rats with EEO significantly prevented the elevation of GOT, GPT, ALP and GGT as compared to CCl_4 only treated group. However, the low dose (250 mg/kg) caused an insignificant decrease in GOT and GGT levels. Silymarin on the other hand, diminished the levels of all marker enzymes as compared to the group treated with CCl_4 only.

3.4. Effect of EEO on lipid profile and bilirubin in serum

The effect of protreatment of rats with ethanolic extract of okra on the CCl_4-induced elevation of the levels of serum cholesterol, triglycerides and bilirubin are shown in Table 2. Administration of CCl_4 significantly elevated the cholesterol, triglycerides and bilirubin contents in serum ($P < 0.001$). Pretreatment of rats with EEO significantly prevented the elevation of cholesterol and triglycerides at both doses used.

Table 1 The effect of EEO on activities of liver marker enzymes in $CC1_4$-induced hepatotoxicity in rats.

Groups	Treatment	Dose (mg/kg, orally)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	GGT (IU/L)
1	Control (distilled water)	–	93.71 ± 13.30	23.86 ± 5.87	351.19.93	3.43 ± 0.57
2	CCl_4[a]		334.66 ± 31.91***	290.83 ± 28.51***	878.83 ± 34.49***	28.51 ± 7.50***
3	Okra extract[b]	250	303.66 ± 23.04	197.16 ± 23.01*	686.16 ± 38.22**	17.61 ± 2.79
4	Okra extract[b]	500	186.66 ± 19.82**	174.83 ± 20.78**	600.50 ± 4.97***	9.80 ± 1.27*
5	Silymarin[b]	10	132.66 ± 20.51***	92.65 ± 16.77***	425.00 ± 27.29***	7.58 ± 1.81*

Data are mean ± SEM. Six rats were used in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's *t*-test.

[a] As compared with control (distilled water) group.

[b] As compared with $CC1_4$ group.

Table 2 The effect of EEO on serum cholesterol, triglycerides and bilirubin levels in CCl_4-induced hepatotoxicity in rats.

Groups	Treatment	Dose (mg/kg, orally)	Cholesterol (g/dl)	Triglycerides (g/dl)	Bilirubin (mg/dl)
1	Control (Distilled water)	–	88.48 ± 11.27	72.98 ± 5.07	0.57 ± 0.06
2	CCl_4[a]		134.83 ± 15.76*	149.33 ± 15.06***	3.30 ± 0.27***
3	Okra extract[b]	250	69.21 ± 8.63**	83.48 ± 12.38**	2.96 ± 0.15
4	Okra extract[b]	500	78.98 ± 10.1*	72.04 ± 12.81**	2.77 ± 0.18
5	Silymarin[b]	10	97.36 ± 16.60	127.16 ± 16.70	1.04 ± 0.16***

Data are mean ± SEM. Six rats were used in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test.
[a] As compared with control (distilled water) group.
[b] As compared with CCl_4 group.

However, EEO in both doses failed to decrease the bilirubin levels. Silymarin could not afford to reduce the cholesterol and triglyceride levels. On the other hand, silymarin significantly diminished the levels of bilirubin as compared to the CCl_4 only treated group.

3.5. Effect of EEO on hepatic MDA

As depicted in Fig. 1, the MDA, an end product of lipid peroxidation, in the rats' liver tissue, treated with CCl_4 was increased when compared with the normal control rats. Pretreatment of rats with EEO resulted in a significant and dose-dependent decrease in the concentration of MDA as compared to the group treated with CCl_4 only. Although low dose (250 mg/kg) of EEO, reduced the MDA level, this decreased level of MDA was not statistically significant. Silymarin treatment significantly reduced the MDA concentration as compared to the group treated with CCl_4 only.

3.6. Effect of EEO on Hepatic NP-SH

Fig. 2 showed the reduced levels of NP-SH caused by CCl_4 treatment, the EEO at both doses used caused significant and dose-dependent elevation of the NP-SH concentration in the liver tissue. Silymarin treatment showed a significantly enhanced NP-SH level as compared to the group treated with CCl_4 only.

3.7. Effect of EEO on hepatic TP

Fig. 3 demonstrated the total protein levels were significantly decreased in CCl_4 only treated group. EEO at both doses used

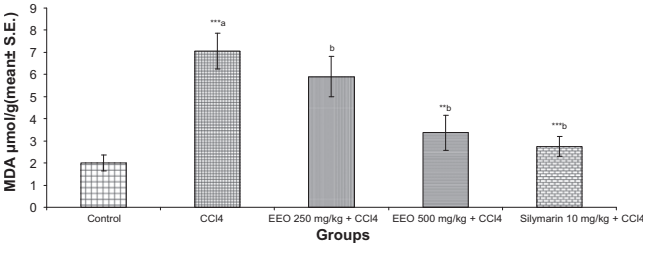

Figure 1 Effect of EEO on liver MDA concentration in CCl_4-induced hepatic injury. All values represent mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ANOVA, followed by Dunnett's multiple comparison test. [a]As compared with normal group. [b]As compared with CCl_4 only group. n = six rats were used in each group.

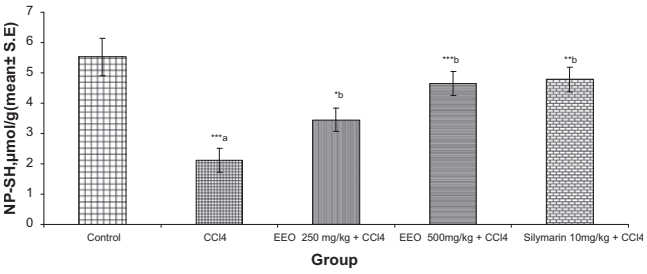

Figure 2 Effect of EEO on liver NP-SH concentration in CCl_4-induced hepatic injury. All values represent mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ANOVA, followed by Dunnett's multiple comparison test. [a]As compared with normal group. [b]As compared with CCl_4 only group. n = six rats were used in each group.

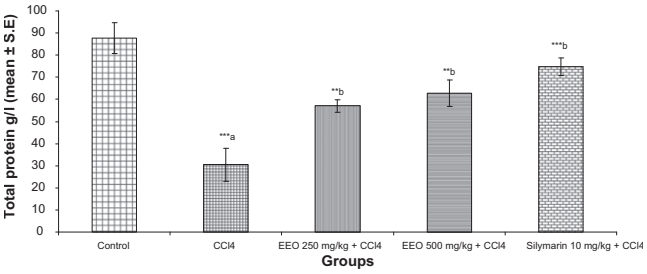

Figure 3 Effect EEO on liver TP concentration in CCl_4-induced hepatic injury. All values represent mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; ANOVA, followed by Dunnett's multiple comparison test. [a]As compared with normal group. [b]As compared with CCl_4 only group. n = six rats were used in each group.

caused significant and dose-dependent elevation of the protein concentration in the liver tissue. Silymarin treated rats also showed a significant increase of total protein as compared to the group treated with CCl_4 only.

3.8. Effect of EER on histopathological evaluation

Histopathological studies provided supportive evidence for the biochemical analysis. The photomicrographs of the liver showed severe necrosis and inflammation in CCl_4 only treated rats (Fig. 4B) in comparison with normal control (Fig. 4A). The EEO treated (250 and 500 mg/kg, p.o.) groups showed minimal inflammation (Figs. 4C and 4D). The silymarin trea-

ted group showed scattered foci of inflammation with the absence of necrosis (Fig. 4E).

3.9. Effect of EEO on pentobarbital-induced sleeping time

There was a significant lowering of pentobarbital-induced sleeping time following the administration of the EEO in the CCl_4-induced acute liver injury model (Table 3).

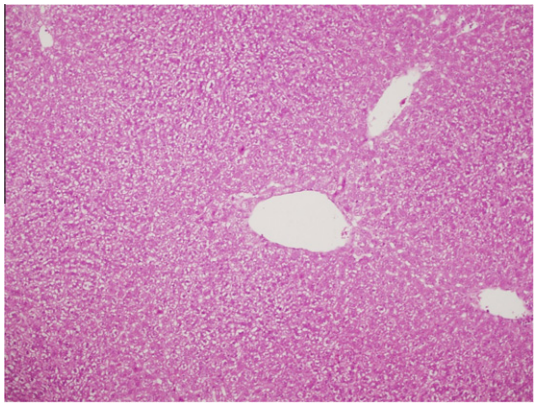

Figure 4A Liver (normal). Normal hepatocytes. H. & E. 200×.

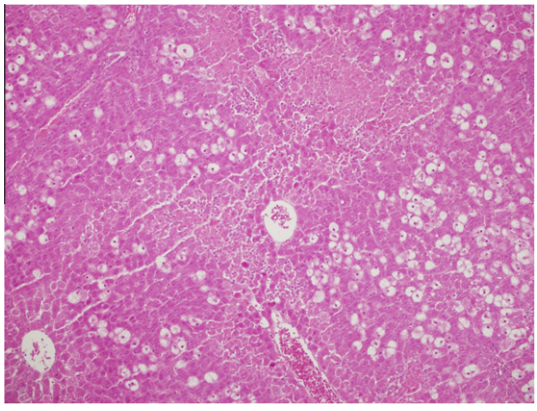

Figure 4B Liver (treated with CCl_4 only). Hepatocytes showed severe necrosis and inflammation. H. & E. 200×.

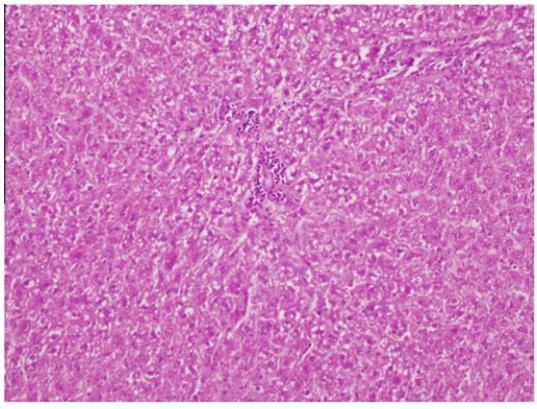

Figure 4C Liver (treated with CCl_4 and 250 mg/kg EEO). Normal hepatocytes. H. & E. 200×.

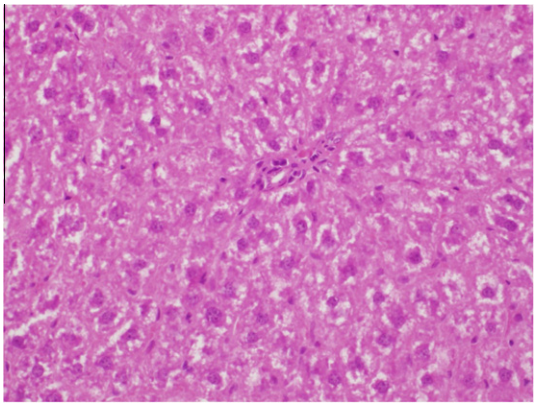

Figure 4D Liver (treated with CCl_4 and 500 mg/kg EEO). Normal hepatocytes. H. & E. 200×.

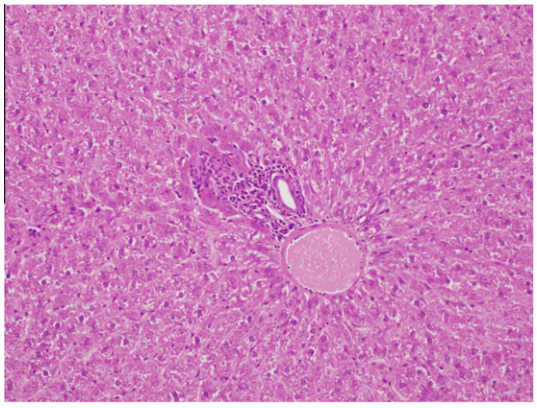

Figure 4E Liver (treated with CCl_4 and 10 mg/kg silymarin). Scattered foci of inflammation with the absence of necrosis. H. & E. 200×.

4. Discussion

The damage to the liver after CCl_4 administration is a well known phenomenon, and the obvious sign to hepatic injury is the leakage of cellular enzymes into the plasma (Recknagel et al., 1989). The experimental intoxication induced by CCl_4 is widely used for modeling liver injuries in rats. Hepatotoxicity is connected with the severe impairment of cell protection mechanisms. The location of liver injury is defined mainly by the biotransformation of CCl_4, as CCl_4 is metabolized by cytochrome P 450 systems to yield trichloromethyl radical ($CCL_3^{\cdot}$) and peroxy radical ($CCl_3OO^{\cdot}$) (Huang et al., 1995), which are highly reactive oxygen species (ROS) and are capable of combining with membrane lipids and proteins in the presence of O_2 to induce lipid peroxidation (LPO) (Kadiiska et al., 2000; Pradeep et al., 2007). In the present study, the elevated levels of serum enzymes such as GOT, GPT, ALP and GGT have been observed in CCl_4-treated rats, which indicate the increased permeability, damage, and/or necrosis of the hepatic cells. The present study demonstrated that orally pre-administered EEO suppressed CCl_4-induced hepatic injury dramatically. EEO was found to have a hepatoprotective effect, as shown by its ability to significantly reduce CCl_4-induced hepatic marker enzymes, cholesterol, triglycerides, and MDA; be-

Table 3 The effect of EEO on duration of pentobarbital sleeping time in mice treated with CCl_4.

Groups	Treatment	Dose	Sleeping time (min)	Reduction in sleeping time (%)
1	Pentobarbital only	(50 mg/kg, i.p.)	77.50 + 9.26	–
2	Pentobarbital + CCl_4[a]	(50 mg/kg, i.p. + 1.5 ml/kg, i.p.)	165.60 + 9.28***	–
3	EEO + CCl_4 + pentobarbital[b]	(250 mg/kg, p.o. + 1.5 ml/kg, i.p. + 50 mg/kg, i.p.)	125.80 + 6.84**	24.03
4	EEO + CCl_4 + pentobarbital[b]	(500 mg/kg, p.o. + 1.5 ml/kg, i.p. + 50 mg/kg, i.p.)	97.20 + 4.57***	41.30

Data are Mean ± SE. Ten mice were used in each group. $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test.
[a] As compared with control (distilled water) group.
[b] As compared with $CC1_4$ group.

sides an elevation in NP-SH and total protein levels. The antihyperlipidemic effect of EEO may be due to the down regulation of NADPH and NADH cofactors in the fat metabolism. EEO may also exert its antilipidemic action by oxidizing NADPH (Fernandes et al., 2007; Al-Dosari et al., 2011).

The elevated level of MDA, which is one of the end products of lipid peroxidation in the liver tissue and diminished levels of total protein and NP-SH are important indicators of tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals in CCl_4-intoxicated animals (Souza et al., 1997). In this study, it was observed that the CCl_4-induced depletion of NP-SH and TP were replenished significantly and the increased MDA levels were suppressed (compared with the CCl_4 only treated group). The EEO pretreatment successfully quenched the free radicals thereby inhibiting lipid peroxidation and protecting the membrane lipids from oxidative damage in the liver of rats (Pradeep et al., 2007). Hence, EEO acts as a potent antioxidative agent under *in vivo* conditions (Arapitsas, 2008) and an earlier report also highlighted the *in vitro* antioxidative effect of okra water extract (Ansari et al., 2005). The treatment of rats with CCl_4 caused damage to microsomal drug metabolizing enzymes in hepatocytes leading to a substantial decrease in hepatic drug metabolizing capacity, being reflected in the prolongation of pentobarbital-induced sleeping time (Rafatullah et al., 1991; Janbaz et al., 2004). The pretreatment of mice with EEO prevented the CCl_4-induced prolongation in pentobarbital-induced narcolepsy, further confirming a protective effect of EEO against CCl_4-induced damage to hepatocytes.

The preliminary phytochemical studies showed that flavonoids, tannins, sterols and triterpenes were present in okra. Phytoconstituents, such as flavonoids are commonly present in vegetables and fruits, which provide the health-benefits, associated with diets rich in plant-food. Flavonoids are a class of secondary plant phenolics, found ubiquitously in fruits, vegetables and medicinal plants (Al-Howiriny et al., 2003; Yazdanparast et al., 2008) which are known to play a pivotal role as dietary antioxidants for the prevention of oxidative damage in the living system (Hertog et al., 1993). Furthermore, a large number of biological actions of flavonoids have been attributed to their potent antioxidant properties; as they act in different ways, including direct quenching ROS, chelation of metal ions and regeneration of membrane-bound antioxidants (Heber, 2004; Sunilson et al., 2008). The obtained results demonstrated that the possible mechanisms of okra extract to protect liver toxicity produced by CCl_4 in rats, might be due to following effects: (i) prevention of lipid peroxidation; (ii) the hepatocyte membrane stabilization; (iii) abolishment or inhibition of the cytochrome P 450-dependent oxygenase activity and (iv) enhancement of non-protein sulfhydryls (NP-SH) and total proteins (TP) concentration in liver tissue possibly due to its antioxidative potential. However, from the data obtained it is concluded that the active phytoconstituents of *Hibiscus esculentus* pods, which are responsible for the obtained liver-protective and antioxidant effects, have been only detected but not isolated in this study and support the claims made by Hakims and Vaids for its usefulness in liver and gastro-intestinal ailments.

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